GENOPLANTE ®

P059

ANR n°: ANR-07-GPLA-017

Acronym: GENESALB

Thematic: Other Species

Total Cost: 1 710 Keuros
Total Grant: 442 Keuros

Date of beginning: 01.01.2008 – End: 31.12.2010

ANR

Edition 2007

# Genetic analysis of resistance to South American Leaf Blight (SALB) in rubber tree (*Hevea* spp.)

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## Aims

*Hevea brasiliensis* cultivation is nearly the only source of production of natural rubber, an irreplaceable strategic commodity upon which are dependent various industrial sectors. The worldwide area of rubber tree cultivation has continuously increased still the beginning of the 20th century, except in South and Central America, where an endemic disease has prevented its development. This foliar disease, called South American Leaf Blight (SALB), is due to the Ascomycota fungus *Microcyclus ulei*. It causes severe foliar decay and can end in the death of trees of the most susceptible cultivars, such as high yielding cultivars from Asia or Africa.

The objective of the present project is to enforce and develop the research investment in molecular genetics of SALB tolerance initiated with the CMB project. It is proposed to combine complementary actions in genetic mapping and in candidate genes identification for speeding the characterization of genetics factors of natural resistance to SALB and ultimately setting a marker-aided selection program.

## Results

QTL analysis of a durable resistance (MDF180) – [subproject 1]

A new resistance to SALB has recently been described in the cultivar MDF180 originated from Peru (Le Guen *et al.* 2008, Crop Prot, 27:1498-1503), lasting for more than 30 years in areas under high inoculum pressure. Resistance of MDF180 to SALB is characterized by the complete absence of sexual phase of the fungus (teleomorph), a limited asexual sporulation, and no foliar decay. Pseudo-testcross QTL mapping was performed on 298 F1 descendants from a Susceptible(PB260)xMDF180 cross, located in French Guiana. On this progeny, we built a genetic map encompassing 256 markers (95 AFLPs and 177 SSRs) and leading to a sufficient coverage of the 18 *Hevea* chromosomes. Surprisingly, the genetic determinism of this durable resistance is relatively simple, being genetically supported mainly by one major gene (linkage group g15), governing resistance to Guianese strains of the fungus, and a strong QTL (linkage group g13), efficient against Brazilian strains.

Fine mapping of a major resistance gene from FX2784 – [subproject 1]

We previously identified and mapped an other major resistance gene, governing the complete resistance of the FX2784 cultivar. Using SSR mapping of 125 F1 individuals located in French Guiana, we mapped this major gene on the linkage group g2. In the present project, we localized more accurately this major gene by SSR mapping on a population of 295 additional F1 individuals from FX2784, evaluated for SALB resistance in Brazil (Michelin PEM estate, Mato Grosso). The results confirm that the same locus explain the complete resistance in the both locations (French Guiana and Brazil) and the mapping results are highly congruent. Two SSR markers flank the resistance locus at 5 cM.

Candidate genes identification – [subproject 3]

Candidate genes (ESTs) were first identified and cloned using SSH approach based on "Susceptible *vs* Resistance" or "Inoculated *vs* Non-inoculated" subtraction. A sub-set of candidate genes was then defined among the 6992 cloned SSH-unigenes using expression analysis by macroarrays hybridizations. Three hundred eighty two ESTs, over- or under-expressed during infection, were retained for further map integration.

EST-SSRs identification and mapping – [subproject 2]

We apply several genotyping methods for polymorphic genetic marker development and candidate ESTs mapping. First of all, we search for SSR repeats (di- to hexa-nucleotide repeats) in the whole set of unigenes using the ESTtik software. We found 287 ESTs with at least 1 SSR motif. Genetic polymorphism and mapping were carried out on 54 EST-SSRs using the bin-mapping method.

**Perspectives**

During the third and last year of the project (2010), the following research activities will be carried out:

a) Genetic mapping – [subproject 1]: SSR mapping of 2 additional progenies located at the Michelin PMB estate (Bahia, Brazil: PB260xMDF180 & WxFDR5597).

b) QTL mapping and diversity of genetic determinisms – [subproject 1]: field evaluation of SALB resistance for 3 progenies in Brazil (PB260xFX2784; PB260xMDF180; WxFDR5597); SALB resistance evaluation in controlled conditions of 1 fourth progeny in French Guiana (PB260xRO38); QTL mapping finalized at the end of 2010, when complete phenotypic data available

c) Candidate genes characterization – [subproject 3]: expression analysis by Q-PCR of a subset of candidate ESTs

d) Genetic markers development and candidate genes mapping in 4 progenies – [subproject 2]: achievement of EST-SSRs mapping; EST-SNPs identification and mapping using HRM genotyping method; development of genetic markers from BAC clone sequences, corresponding to candidate genes and/or to chromosome regions containing major genes/QTLs.

**Publications / Congress**

Publications (in prep.)

1. Le Guen V., Garcia D., Doaré F., Condina V., Couturier C., Weber C., Espéout S., Seguin M. Genetic mapping for major gene and QTLs of resistance to South American Leaf Blight due to *Microcyclus ulei*, in a highly resistant rubber tree cultivar.
2. Garcia D., Carels N., de Sousa L.A, Sizenando Andrade S.J., Pujade-Renaud V., Mattos C.R.R., Cascardo J.C.M. Profiling of genes from *Hevea brasiliensis* involved in the resistance to *Microcyclus ulei*.

Congress

1. Berger A, Déon M, Doaré F, Goujon E, Garcia D, Seguin M, Pujade-Renaud V (2010). Identification de gènes candidats impliqués dans les processus de résistance de l'hévéa à *Microcyclus ulei* par la technique des macroarrays. *In: Journées Jean Chevaugeon*, Aussois, 25-29 Janv 2010. Poster
2. Le Guen V, Garcia D, Doaré F, Weber C, Chambon A, Seguin M (2009) Natural pyramiding provides rubber tree with durable resistance to South American Leaf Blight. *In: Plant-GEM 8*, Lisbon 2009, 7-10 October 2009, Lisbon, Portugal. Poster n° S2.P.16, abstract p.94.
3. Koop D.M., Conceição L., Cardoso S.E.A., de Sousa L.A., Silva D. da C., Garcia D. (2009) Estudo histológico e molecular da morte celular programada (PCD) na interação *Hevea – Microcyclus ulei*. *In: XLII Congresso Brasileiro de Fitopatologia*. Rio de Janeiro (Brasil). 3 a 7 agosto de 2009. Tropical Plant Pathology. Vol. 34. S257. Poster 868.
4. Argout X., Garcia D., Montoro P., Pujade-Renaud V., Ruiz M., Seguin M., Sidibé Bocs S. (2009). Statement of transcriptomics and bioinformatics analyses conducted at CIRAD in rubber tree: towards the genome analysis. *In: Hevea genome and transcriptome*. *IRRDB Workshop on Hevea Genome and Transcriptome*. 2009/06/03-05, Montpellier, France. Book of abstracts. Montpellier, France: Cirad, IRRDB, IFC, p.49.
5. Garcia D., Carels N., Araújo L.d.S., Koop D.M., Pujade-Renaud V., Silva D.d.C., Mattos C.R.R., Cascardo J.C.M. (2009). Transcriptome comparison of resistant and susceptible *Hevea brasiliensis* cultivars infected by *Microcyclus ulei*. *In: Hevea genome and transcriptome*. *IRRDB Workshop on Hevea Genome and Transcriptome.* Book of abstracts. Montpellier, France. Cirad, IRRDB, IFC, p. 30-48. 2009/06/03-05, Montpellier, France.
6. Seguin M, Argout X, Cavaloc E, Doaré F, Espéout-Fois S, Fonseca F, Garcia D, Granet F, Le Guen V, Mattos C, Pujade-Renaud V, Weber C (2008) GENESALB: Genetic analysis of resistance to South American Leaf Blight – SALB (*Microcyclus ulei*) in rubber tree (*Hevea* spp.). *In: Séminaire Génoplante 2008*. 1-3 Avril 2008, 1p. Arles, France. Poster

Academic training / reports

1. Masters :

- Déon Marine (2008) « Expression analysis by macro-arrays hybridization ». Montpellier University UM2, France
- Bouchata Karima (2009) « EST-SSR mapping ». Blaise Pascal University, Clermont-Ferrand, France.
- Goujon Eric (2009) « Expression analysis using Q-PCR »Blaise Pascal University, Clermont-Ferrand, France.

2. PhD :

- Le Guen Vincent (2008) « Resistance QTL mapping » Montpellier Univ. UM2 & Montpellier SupAgro, France

**Total permanent scientist**

Montpellier: 2.6 EFTs ; Clermont-Ferrand: 1.6 EFTs ; French Guiana: 0.6 EFTs ; Brazil(researchers only): 1.5 EFTs

**Temporary contracts**

| | |
|---|---|
| **Mr Philippe CUBRY**, | Michelin post-doctoral fellow, 18 months, Montpellier from April 1, 2008, to September 30, 2009. |
| **Mrs Sandra ESPEOUT**, | Michelin technician, 18 months, Montpellier from July 1, 2009, to December 31, 2010 |

Plant Genomics Seminar 2010, 2010/03/29-31, Pont-Royal en Provence, France


(photo: M. Seguin)

# GENESALB (2008-2010):
# Genetic analysis of resistance to South American Leaf Blight (SALB, *Microcyclus ulei*) in rubber tree (*Hevea* spp.)


Defoliation due to *M. ulei* in Bahia
(photo: M. Seguin)

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## Introduction

***Hevea brasiliensis* cultivation is nearly the only source of production of natural rubber (latex)**, an irreplaceable strategic bio-polymer for various industrial sectors. Worldwide production is threaten by the **South American Leaf Blight (SALB) due to the fungus *Microcyclus ulei* (Ascomycota)**. CIRAD and Michelin collaborate, since 1992, on a program (CMB, Cirad-Michelin-Brésil) aiming at the **creation of new varieties, combining high latex yield and tolerance to SALB**. The ultimate objective is both **to allow rubber farming development** in the American inter-tropical zone endemically affected by the disease, and **to prevent the risk of accidental introduction** of the pathogen in the current Asian and African producing regions.

The aim of the present project is to reinforce **genetic mapping and candidate genes identification** in order to speed up the **characterization of genetics factors of natural resistance to SALB** and, ultimately, to set up a marker-aided selection program.

## Material & Methods

**Mapping populations**: [SxR] F1 segregating populations: 351 [PB260xMDF180] individuals located in French Guiana; 295 [PB260xFX2784] in Mato Grosso (Brazil).

**Resistance evaluation**: natural (field) or controlled infestations (with isolated strains of *M. ulei*) in French Guiana; Resistance parameters [1][2]: 3 parameters based on intensity of necrosis/chlorosis, conidia sporulation and stromata formation (**Fig. 1**)

**Molecular markers for mapping**: genomic SSRs, AFLPs[1][3][4]

**Mapping strategy**: bin-mapping and pseudo-testcross (*H. brasiliensis*: 2n=2x=36)

**Candidate gene identification**: Susceptible(S) *vs* Resistant(R) EST-SSH libraries: sequence analysis (ESTtik pipeline[5]); expression analyses at different hours after controlled infestations (hpi) through macroarrays hybridization; signal quantification (ImageQuant-TL software) and non-parametric normalization[6].

**Candidate gene genotyping for mapping**: EST-SSRs from SSH clones

A)

(photos: M. Seguin)

B)


C)


Figure 1*: M. ulei* symptoms on *Hevea* adult leaves: A) necrosis, B) conidian sporulation, C) stromata (perithecia containing ascospores)

**S inoc: susceptible (PB260) inoculated**

**R inoc: resistant (RO38) inoculated**

(photos: F. Doaré)

| SSH library | Subtractive combination (tester – driver) | Expected expression of the cloned ESTs during infestation |
|---|---|---|
| A | S inoc – R inoc | S > R |
| B | R inoc – S inoc | R > S |
| E | R inoc – R control | ↗ in R |
| F | R control – R inoc | ↘ in R |

**Figure 2**: Four SSH libraries spotted on macroarray filters and hybridized with $^{33}P$ labelled total leaf cDNAs from PB260(S) or RO38(R) cultivars inoculated or not with a *M. ulei* isolate.

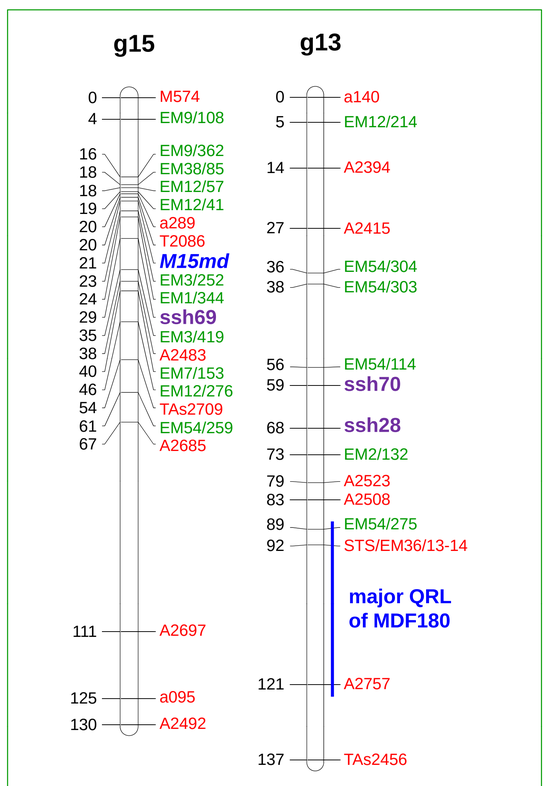


**Figure 4** : Two linkage groups of the MDF180 parental map. Genetic markers: gSSRs, AFLPs, EST-SSRs, SALB resistance loci

## Results (2008-2009)

**Candidate gene identification:** 6 EST-SSH libraries of PB260 *vs* RO38 (S *vs* R) produced and sequenced (Sanger) -> **5428 unigenes**. Macroarrays analysis of 4/6 libraries (**Fig. 2**) -> identification of **394 candidate genes** differentially expressed in S and R cultivars at 48 and 24 hpi (**Fig. 3**). UESC (D. Garcia) provided data from similar analyses of 5 EST-SSH libraries from 2 other cultivars (PB314(S) *vs* MDF180(R)[7]) -> final identification of **674 candidate genes** from **6992 unigenes**.

**Candidate gene mapping:** identification of 282/6992 ESTs containing SSRs -> 142 EST-SSRs analyzed by bin-mapping -> **70 (50%) of EST-SSRs polymorphic and bin-mapped** -> 19 mapped on the complete PB260xMDF180 progeny (**Fig. 4**).

**QTL/mapping:** 1) "fine" mapping of a major SALB resistance gene from the FX2784 cultivar: gSSR mapping and phenotyping of the population from Mato Grosso -> same *M2fx* locus than previously identified in French Guiana**: mapped on linkage group (LG) g2 flanked by 2 gSSRs at 5cM** (data not shown);

2) QTL mapping of PB260xMDF180: 159 SSRs, 96 AFLPs, 14 EST-SSRs mapped in MDF180 parental map -> **18 LG (saturated map)** -> identification of **one major Resistance QTL** on LG g13 and of a **major Resistance gene** (*M15md*) on g15 (**Fig. 4**). After *M13-1bn* on LG g13[2] and *M2fx* (g2), *M15md* is the third major SALB Resistance gene identified in rubber tree.

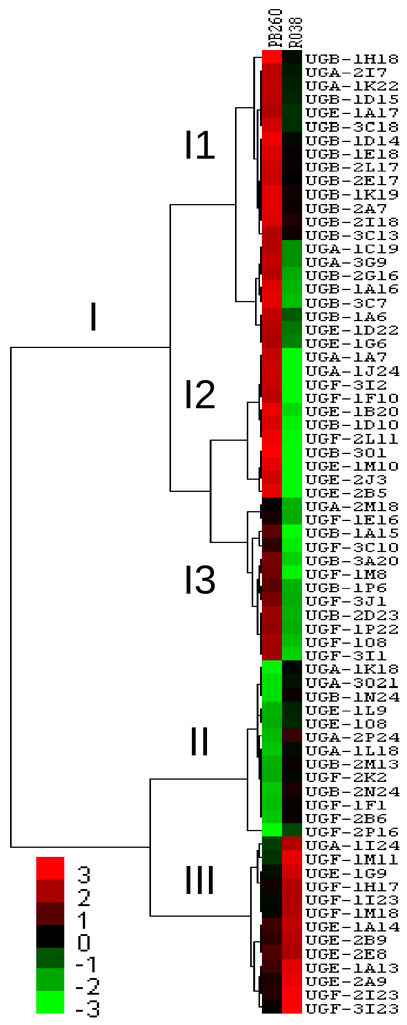


**Figure 3**: Cluster analysis of expression /macroarray data: classification tree obtained for the 71 SSH-ESTs differentially expressed between PB260/S and RO38/R at 24 hpi.

## Prospects

**QTL mapping of other resistance sources:** 1) 171 additional [PB260 x MDF180] F1 in Bahia; 2) 269 [S x FDR5597(R)] F1 in Bahia; 3) 312 additional [PB260 x RO38] F1 in French Guiana[2]

**Candidate gene characterization:** expression analysis of Candidate Genes by Q-PCR

**Candidate gene mapping:** SNPs identification and genotyping by Sanger sequencing and HRM; gSSR identification in BAC clone sequences

## Publications and communications

**Academic courses:** 3 MScD reports (2008-2009) and 1 PhD report (2008)

**Communications:** 4 posters and 2 oral communi-cations in 2 international and 2 national congress


(photo M. Seguin)

Cited references:
[1]. Lespinasse D, Grivet L, Troispoux V, Rodier-Goud M, Pinard F, Seguin M (2000) **Theor. Appl. Genet.** 100: 975-984.
[2]. Le Guen V, Garcia D, Mattos C.R.R, Doaré F, Lespinasse D, Seguin M (2007) **New Phytol.** 173(2), 335-345.
[3]. Seguin M, Gay C, Xiong T-C, Rodier-Goud M (2002) ***In: Proc.of the IRRDB Symp. 2001,*** *Biotech.& Rubber Tree*. 6p.
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